INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

DERIVATION AND CHARACTERISATION OF CELL SUBSTRATES USED FOR PRODUCTION OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q5D

Current *Step 4* version

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This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

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ICH Harmonised Tripartite Guideline

Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on 16 July 1997, this guideline is recommended for adoption to the three regulatory parties to ICH

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1. INTRODUCTION

1.1 Objective

The objective of this guideline is to provide broad guidance on appropriate standards for the derivation of human and animal cell lines and microbial cells to be used to prepare biotechnological/biological products defined in *Section 1.3, Scope*, and for the preparation and characterisation of cell banks to be used for production. The document, therefore, provides recommendations on the information in these areas that should be presented in market applications for these products.

1.2 Rationale

Historically, some quality concerns for cell-derived biological products have originated from the presence of adventitious contaminants or from the properties of the cells used to prepare the product. Recombinant DNA (rDNA) - derived products also carry quality concerns regarding the expression construct contained in the cell substrate. Thus, it is well established that the properties of the cell substrate and events linked to the cell substrate can affect resultant product quality and safety and, further, that effective quality control of these products requires appropriate controls on all aspects of handling the cell substrate.

This document complements other guidelines to provide a comprehensive approach to quality issues arising from biological aspects of processing products from metazoan and microbial cell culture.

1.3 Scope

This guideline covers cell substrates having a cell banking system. In this document, "cell substrate" refers to microbial cells or cell lines derived from human or animal sources that possess the full potential for generation of the desired biotechnological/biological products for human *in vivo* or *ex vivo* use. Reagents for *in vitro* diagnostic use are outside the scope of this document. Animal sources of cell lines include all those of metazoan origin. Both continuous cell lines of indefinite *in vitro* lifespan and diploid cells of finite *in vitro* lifespan are included. Microbial sources include bacteria, fungi, yeast, and other unicellular life forms.

"Biotechnological/biological products" refers to any products prepared from cells cultivated from cell banks with the exception of microbial metabolites such as, for example, antibiotics, amino acids, carbohydrates, and other low molecular weight substances. Cell banks used to prepare gene therapy products or vaccines should follow the recommendations presented in this document. Some biological products, such as certain viral vaccines, are prepared in primary cell cultures derived directly from animal tissues or organs. Primary cells are not banked and therefore are not addressed by this document. However, other considerations which may apply to primary cells are discussed further in Appendix 1 of this document.

2. GUIDELINES

2.1 Source, History, and Generation of the Cell Substrate

2.1.1 Introduction

It is important to provide supportive documentation which describes the history of the cell substrate that is used in the manufacture of a biotechnological/biological product, as well as any parental cell line from which it was totally or partially derived. Events during the research and development phases of the cell substrate may contribute significantly to assessment of the risks associated with the use of that particular cell substrate for production. The information supplied in this regard is meant to facilitate an overall evaluation which will ensure the quality and safety of the product.

Careful records of the manipulation of the cell substrate should be maintained throughout its development. Description of cell history is only one tool of many used for cell substrate characterisation. In general, deficiencies in documented history may not, by itself, be an impediment to product approval, but extensive deficiencies will result in increased reliance on other methods to characterise the cell substrate.

2.1.2 Origin, Source, and History of Cells

The source of cells (laboratory or culture collection) from which the cell substrate was derived should be stated, and relevant references from the scientific literature should be cited. Information obtained directly from the source laboratory is preferred. When this is not available, literature references may be utilised.

For human cell lines, it is relevant to describe the following characteristics of the original donor: Tissue or organ of origin, ethnic and geographical origin, age, sex and general physiological condition. If known, the state of health or medical history of the donor should be reported along with the results of any tests of the donor for pathogenic agents. Specifically for human diploid fibroblasts, the age of the donor may influence the *in vitro* lifespan of the cell line and this information should be provided if available. For animal cell lines, relevant descriptions of the source include species, strains, breeding conditions, tissue or organ of origin, geographical origin, age and sex, the results of tests for pathogenic agents, and general physiological condition of the original donor.

For microbes, manufacturers should describe the species, strain, and known genotypic and phenotypic characteristics of the organism from which the cell substrate was derived. Manufacturers should also describe the pathogenicity, toxin production, and other biohazard information, if any.

The cultivation history of the cells should be documented. The method originally used for the isolation of the cells should be described as well as the procedures used in the culturing of the cells *in vitro* and any procedures used to establish cell lines (for example, use of any physical, chemical, or biological procedure, or added nucleotide sequences). A description of any genetic manipulation or selection should be provided. All available information regarding the identification, characteristics, and results of testing of these cells for endogenous and adventitious agents should be provided.

For continuous cell lines of metazoan origin, it is usually adequate to quantitate culture duration by estimation of either number of population doublings, or number of subcultivations at defined dilution ratio, or time in days. For diploid cell lines

possessing finite *in vitro* lifespan, accurate estimation of the number of population doublings during all stages of research, development, and manufacturing is important. For microbial cells, documentation of subcultivation frequency after cell substrate generation is considered adequate.

Regarding the generation of cell substrates, applicants should provide a thorough discussion of procedures which would provide exposure to infectious agents. Constituents of the culture medium should be described, in particular, information regarding exposure of the cells to materials of human or animal origin such as serum, enzymes, hydrolysates, or other living cells. The description should include the source, method of preparation and control, test results, and quality assurance. Relevant literature on these points may be referenced when available. This information will allow a detailed analysis of potential entry routes for adventitious agents from these sources, and will be part of the risk-benefit analysis of the product.

2.1.3 Generation of the Cell Substrate

A crucial step is the choice of a suitable parental cell line. For recombinant products, a parental cell line is typically the untransfected recipient cell line. The use of characterised parental cell banks is suggested, but is not considered essential. A characterised parental cell bank may be of benefit, especially when multiple cell substrates are generated from the same parental cell type, by providing a set of information on which the quality assessment of the Master Cell Bank (MCB) can be based. For example, the myeloma cell line may be banked as a parental cell line for hybridomas.

During the generation of the cell substrate, one or more specific procedures may be utilised in the ultimate development of the desired characteristics. These may include, for example, cell fusion, transfection, selection, colony isolation, cloning, gene amplification, and adaptation to specific culture conditions or media. Information regarding the methodologies utilised in developing the cell substrate can help to provide a clear understanding of the history of the cell substrate. Some cell substrates such as human diploid fibroblasts may not need extensive manipulation or cloning prior to cell banking.

For recombinant products, the cell substrate is the transfected cell containing the desired sequences, which has been cloned from a single cell progenitor. For further information on generation of rDNA-modified cell substrates, consult other relevant (e.g., regional or international) guidelines. For non-recombinant products or non-recombinant vaccines, the cell substrate is the cell from the parental cell line chosen for preparation of the MCB without further modification. For products derived from hybridomas, the cell substrate is the hybridoma cell line derived by fusion of the parental myeloma cell line with other parental cells, e.g., immune spleen cells.

2.2. Cell Banking

One of the most important advantages of using serially subcultivated cells to produce biotechnological/biological products is the ability to have a characterised common starting source for each production lot, i.e., the preserved bank of cells. Manufacturers may prepare their own cell banks, or may obtain them from external sources. Manufacturers are responsible for ensuring the quality of each cell bank and of the testing performed on each bank.

2.2.1 Cell Banking System

The concept of a two-tiered cell bank, in which the MCB which is used to generate Working Cell Banks (WCBs), is generally accepted as the most practical approach to providing a supply of cell substrate for continued manufacture of the product. Manufacturers should describe their strategy for providing a continued supply of cells from their cell bank(s), including the anticipated utilisation rate of the cell bank(s) for production, the expected intervals between generation of new cell bank(s), and the criteria for qualification of cell bank(s).

Generally, the MCB is made first, usually directly from an initial clone or from a preliminary cell bank derived from an initial clone. It is not considered necessary to prepare cell banks from clones for certain types of cells (e.g., diploid cells, where limited *in vitro* life span or other technical factors make cell cloning impractical) or where the uncloned cell population is already adequately homogeneous for the intended use.

A WCB is derived from one or more containers of the MCB. It is the WCB which is typically used to directly provide cells for the manufacturing process. Additional WCBs are generated from the MCB as needed. A newly prepared WCB should be appropriately qualified by characterisation and testing.

It should be noted that the MCB and WCB may differ from each other in certain respects, e.g., culture components and culture conditions. Similarly, the culture conditions used to prepare the MCB and WCB may differ from those used for the production process. If changes in cell culture process do not affect product quality, it is not considered necessary to reclone the cells or to rebank the MCB or WCB. It is important that a characterised bank provides a consistent product.

A single-tiered banking system consisting only of the MCB but no WCBs could be used in principle, for example, if relatively few containers were needed each year to produce the desired product.

In some microbial expression systems, a new transformation is performed for each new cell substrate container lot, based upon using aliquots of thoroughly tested host cell banks and plasmid banks for each new transformation and on testing of each transformed cell substrate bank. This transformed cell substrate bank is considered the MCB, and it is used as the source of cell substrate for production. Host cell banks, plasmid banks, and MCBs are maintained by appropriate preservation methods. This alternative system is considered adequate because the transformation of bacteria and yeast is generally a very reproducible and easily performed process, unlike the events needed for transfection of metazoan cells. Manufacturers should provide information on the host cells, rDNA molecules (such as plasmids), method of transformation and of cell banking, and the results of characterisation studies.

2.2.2 Cell Banking Procedures

It is important to prevent a contaminated cell substrate (or bank) from being used in production and to avoid a loss of product availability or development time resulting from the need to recreate a cell bank found to be unusable due to contamination. It is recognised that no cell bank testing regimen is able to detect all potential contaminants; therefore, use of these preventive principles during cell banking is important to provide reasonable assurance of the absence of contamination and to provide a reliable source of the cell substrate.

Manufacturers should describe the type of banking system used, the size of the cell bank(s), the container (vials, ampoules, or other appropriate vessels) and closure system used, the methods used for preparation of the cell bank(s) including the cryoprotectants and media used, and the conditions employed for cryopreservation and storage.

Manufacturers should describe the procedures used to avoid microbial contamination and cross-contamination by other cell types present in the laboratory, and the procedures that allow the cell bank containers to be traced. This should include a description of the documentation system as well as that of a labelling system which can withstand the process of preservation, storage, and recovery from storage without loss of labelling information on the container.

Manufacturers should describe their cell banking procedures. Cells are generally prepared for banking by expanding cultures in a progressively greater number or larger size of vessel until a pool of cells can be obtained which is sufficient to generate enough containers for the bank. To ensure the uniform composition of the contents of each container, a single pool of cells for banking should be prepared by combining the cells from all of the culture vessels, if more than one vessel is used.

Cells suspended in preservation medium are aliquoted from the single pool into sterilised containers which are then sealed and stored under appropriate conditions. For example, animal cells in media containing a cryoprotectant are frozen in the sealed containers under defined and controlled conditions, and then transferred to storage in the vapor or liquid phase of liquid nitrogen or at equivalent ultra low temperatures. Other methods of preservation and storage may be adequate depending on the organism used, but they should be capable of maintaining a level of cell viability upon reconstitution which is both consistent and adequate for production use.

To ensure continuous, uninterrupted production of pharmaceuticals, manufacturers should carefully consider the steps that can be taken to provide for protection from catastrophic events that could render the cell bank unusable. Examples of these events include fires, power outages and human error. Manufacturers should describe their plans for such precautions; for example, these may include redundancy in the storage of bank containers in multiple freezers, use of back-up power, use of automatic liquid nitrogen fill systems for storage units, storage of a portion of the MCB and WCB at remote sites, or regeneration of the MCB.

The starting point of reference for estimates of *in vitro* cell age during manufacturing should be the thawing of one or more containers of the MCB. For diploid cell lines, *in vitro* lifespan should be estimated in terms of population doubling levels. The population doubling level at which senescence occurs should be determined for diploid cells.

2.3. General Principles of Characterisation and Testing of Cell Banks

The characterisation and testing of banked cell substrates is a critical component of the control of biotechnological and biological products. Characterisation of the MCB allows the manufacturer to assess this source with regard to presence of cells from other lines, adventitious agents, endogenous agents and molecular contaminants (e.g., toxins or antibiotics from the host organism). The objective of this testing is to confirm the identity, purity, and suitability of the cell substrate for manufacturing use. In some cases, additional testing such as tumorigenicity or karyology may be

useful. The testing program chosen for a given cell substrate will vary according to the biological properties of the cells (for example, growth requirements), its cultivation history (including use of human-derived and animal-derived biological reagents) and available testing procedures. The extent of characterisation of a cell substrate may influence the type or level of routine testing needed at later stages of manufacturing. Manufacturers should perform tests for identity and purity once for each MCB, and tests of stability during cell cultivation once for each product to be registered. In addition, tests of purity and limited tests of identity should be performed once on each WCB. Also, applicants should consult the ICH guideline on viral safety. Relevant tests among those described below should be performed and described in the market application, along with the results of the testing.

For cell lines containing exogenously assembled expression constructs, the relevant ICH guideline on rDNA expression constructs should be consulted for guidance on the characterisation of nucleotide and amino acid sequences. It may also be useful to examine, by similar methods, the coding sequences in some non-recombinant DNA-derived cell lines where the gene sequences have been characterised and are well understood. However, it is not considered necessary to carry out investigations of the sequences encoding complex natural products, for example, families of related gene products, microbial vaccine antigens, or monoclonal antibodies from hybridomas.

Manufacturers are also encouraged to employ "state-of-the-art" methods and technological improvements in cell substrate characterisation and testing as they become available, as long as the specificity, sensitivity, and precision of the newer methods are at least equivalent to those of existing methods.

The manufacturer may choose to characterise the WCB instead of the MCB, if justified.

2.3.1. Tests of Identity

Appropriate tests should be performed to determine that the banked cell is what it is represented to be. Either phenotypic or genotypic characteristics may be used in identity testing. It is not considered necessary to do all the possible tests. Tests of identity are generally performed on the MCB. In addition, limited identity testing is generally performed on each WCB.

2.3.1.1 Metazoan Cells

For human or animal cells which grow attached to a substratum, morphological analysis may be a useful tool in conjunction with other tests. In most cases, isoenzyme analysis is sufficient to confirm the species of origin for cell lines derived from human or animal sources; other tests may be appropriate depending on the history of the cell line. Other technologies may be substituted to confirm species of origin, including, for example, banding cytogenetics or use of species-specific antisera. An alternative strategy would be to demonstrate the presence of unique markers, for example, by using banding cytogenetics to detect a unique marker chromosome, or DNA analysis to detect a genomic polymorphism pattern (for example, restriction fragment length polymorphism, variable number of tandem repeats, or genomic dinucleotide repeats). Either confirmation of species of origin or presence of known unique cell line markers is considered an adequate test of identity. Expression of the desired product may represent a complementary approach to confirmation of identity.

2.3.1.2 Microbial Cells

For most microbial cells, analysis of growth on selective media is usually adequate to confirm host cell identity at the species level for the host cell bank and the transformed cell bank. For *E. coli,* where a variety of strains may be used, biological characterisation methods such as phage typing should be considered as supplementary tests of identity. For plasmid banks, identity assessment can be accomplished as described by the ICH document on analysis of the expression construct. Expression of the desired product is also considered adequate to confirm the identity of the microbial expression system.

2.3.2 Tests of Purity

A critical aspect of cell development and banking is the assessment that the MCB and WCB are biologically pure, i.e., are free from adventitious microbial agents and adventitious cellular contaminants. The impact of selective agents and antibiotics on the detection of adventitious microbial contaminants should be considered when planning and performing these tests.

2.3.2.1 Metazoan Cells

Tests for the presence of bioburden (bacteria and fungi) should be performed on individual containers (1% of the total number but not less than two containers) of the MCB and WCB. In all other aspects, the current methodologies described in either the European Pharmacopoeia (Ph. Eur.), the Japanese Pharmacopoeia (JP) or the U.S. Pharmacopoeia (U.S.P.) for testing microbial limits or microbial sterility may be considered adequate.

Tests for the presence of mycoplasma should be performed on the MCB and WCB. Current procedures considered adequate include both the agar and broth media procedures as well as the indicator cell culture procedure. Current methods for mycoplasma testing are described in Ph. Eur., JP, and "Points to Consider in the Characterisation of Cell Lines Used to Produce Biologicals" (FDA, CBER, 1993). Testing cells derived from a single container is generally considered adequate. For non-mammalian animal cell lines, alternative controls and/or assay conditions may be appropriate; manufacturers should consult with the national/regional regulatory authority for appropriate methodology.

If future efforts to harmonize bioburden and mycoplasma assays are fruitful, then the scientifically appropriate harmonized assay should be used.

Virus testing of cell substrates should be designed to detect a wide spectrum of viruses by using appropriate screening tests and relevant specific tests, based on the cultivation history of the cell line, to detect possible contaminating viruses. Applicants should consult the ICH guideline on viral safety. For product classes not covered by the viral safety guideline, the current World Health Organization (WHO) documents for use of animal cells may be consulted.

The purity of cell substrates can be compromised through contamination by cell lines of the same or different species of origin. The choice of tests to be performed depends upon whether opportunities have existed for cross-contamination by other cell lines. In some cases, it may be necessary to maintain growing cultures of different cell lines in the same laboratory. During procedures in cell banking where open manipulations are performed, care should be taken to ensure that simultaneous open manipulations of other cell lines are avoided to prevent cross-contamination. Whenever another cell line was present in the cell banking room at the same time that open cell banking

procedures were being performed (such as cell expansion, pooling, or aliquoting of the chosen cell line), the cell banks should be tested for the presence of cells from (or products derived from) the second cell line. In general, the methods described in *Section 2.3.1* to assess cell identity are also considered adequate tests to detect cross-contamination by other cell lines. Additional assurance of lack of cross-contamination can be provided by successful preparation of the intended product from the cell substrate.

2.3.2.2 Microbial Cells

The design and performance of specific tests for adventitious microbial agents and adventitious cellular contaminants in microbial cell banks should take into account the properties of the banked cell, the likely contaminants based upon scientific literature, source, methods and materials used for cultivation, and other organisms present in the banking laboratory. For example, visual examination of the characteristics of well-isolated colonies is suggested, using several microbiological media, of which some do and some do not support growth of the cell substrate. However, it is not intended that manufacturers necessarily characterise resistant mutants of the cell substrate arising from such studies, or other artifacts of such assays. Rather, the purpose of such assays is to detect existing contaminants.

2.3.3 Cell Substrate Stability

Another dimension to cell characterisation is appropriateness for intended use in production. There are two concerns for cell substrate stability: Consistent production of the intended product and retention of production capacity during storage under defined conditions.

For the evaluation of stability during cultivation for production, at least two time points should be examined, one using cells which have received a minimal number of subcultivations, and another using cells at or beyond the limit of *in vitro* cell age for production use described in the marketing application. The limit of *in vitro* cell age for production use should be based on data derived from production cells expanded under pilot plant scale or commercial scale conditions to the proposed limit of *in vitro* cell age for production use or beyond. Generally, the production cells are obtained by expansion of cells from the WCB; cells from the MCB could be used with appropriate justification. This demonstration of cell substrate stability is commonly performed once for each product marketing application.

Evaluation of the cell substrate with respect to the consistent production of the intended product of interest should be the primary subject of concern. The type of testing and test article(s) used for such assessments will depend on the nature of the cell substrate, the cultivation methods, and the product. For cell lines containing recombinant DNA expression constructs, consistency of the coding sequence of the expression construct should be verified in cells cultivated to the limit of *in vitro* cell age for production use or beyond by either nucleic acid testing or product analysis, as described in the relevant ICH guideline. For non-recombinant cell lines in which the coding sequence for the desired product has already been analyzed at the MCB or WCB level, invariability of the protein coding sequence during production should be verified in the production cells cultivated to the proposed limit of *in vitro* cell age for production use or beyond by either nucleic acid testing or analysis of the purified protein product.

Where the product cannot be analyzed as described above, other specific traits which may include, for example, morphological characteristics, growth characteristics, biochemical markers, immunological markers, productivity of the desired product, or other relevant genotypic or phenotypic markers may be useful for the assessment of cell substrate stability. In some cases, where direct comparison of the characteristics of the MCB with those of the production cells at or beyond the limit of *in vitro* cell age is difficult or impossible, one may compare the characteristics of cells at the initial stages of cultivation or production to those of cells at or beyond the limit of *in vitro* cell age for production use in order to assess cell stability during production. Indices such as, for example, oxygen or glucose consumption rates, ammonia or lactate production rates may be useful for such testing. Increases in the defined limit of *in vitro* cell age for production use should be supported by data from cells which have been expanded to the proposed new limit of *in vitro* cell age. For diploid cell lines, data should be presented that establish the finite *in vitro* lifespan of the cells from the WCB under conditions representative of those employed for manufacturing use.

Evidence for banked cell stability under defined storage conditions will usually be generated during production of clinical trial material from the banked cells. Data from the determination of cell viability when the preserved cells are reconstituted for production of clinical trial supplies will verify that the revived cells have survived the preservation process. Data from the preparation of clinical materials will demonstrate that the revived cells can be used to prepare the desired product. Available data should be clearly documented in the application dossiers, plus a proposal for monitoring of banked cell stability should be provided. The proposed monitoring can be performed at the time that one or more containers of the cryopreserved bank is thawed for production use, when the product or production consistency is monitored in a relevant way, or when one or more containers of the cryopreserved MCB is thawed for preparation of a new WCB (and the new WCB is properly qualified), as appropriate. In the case when production does not take place for a long period of time, viability testing on the cell bank used as a source of the production substrate should be performed at an interval described in the marketing application. If the viability of the cell substrate is not significantly decreased, generally no further testing of the MCB or WCB is considered necessary.

2.3.4 Tests for Karyology and Tumorigenicity

Utilisation of karyology and tumorigenicity testing for evaluating the safety of a diploid cell line or characterizing a new cell line may be useful depending on the cells, the nature of the product and the manufacturing process. Extensive analysis to determine the relative abundance of aneuploid cells has not been found to be useful. Karyology need not be determined for rodent cell lines or new cell lines known to be non-diploid. However, cytogenetic analysis may be an adequate method to assess cell substrate identity or purity as described in *Sections 2.3.1* and *2.3.2*. Repetition of tumorigenicity testing for cells with already documented evidence of tumorigenicity is not considered necessary.

For products that are highly purified and that contain no cells, karyology and tumorigenicity testing are generally not considered necessary, provided that appropriate limits for residual host cell DNA are shown to be consistently met by either process validation studies or by lot release testing.

In general, products for which the presence of live cells cannot be excluded or which have little downstream purification (for example, some conventional live virus vaccines) will need such characterization of the cell substrate. The utility of tumorigenicity testing and chromosomal analysis for new cell substrates for

unpurified products should be evaluated on a case-by-case basis. Use of cell lines known to be tumorigenic or to possess abnormal karyology should be evaluated in terms of risk-benefit for each product application when the product contains cells or when not highly purified.

Products that are manufactured in genetically unmodified MRC-5 or WI-38 cells do not need characterization of these cell substrates by karyology or tumorigenicity since extensive characterization has already been performed and published for these cell lines. However, for each MRC-5 and WI-38 WCB generated, manufacturers should confirm, once, that the cells grown in the manner to be used in production are diploid and have the expected lifespan.

For new or previously uncharacterized diploid cell substrates, confirmation of diploid karyology should be presented and tumorigenic potential should be established, using cells from the MCB. Methods for karyological and tumorigenicity analysis may be found in the WHO document "WHO.Requirements for Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals" in *WHO Expert Committee on Biological Standardization, 47th Report, Geneva, World Health Organization* (WHO Technical Report Series, in press).

3. GLOSSARY

Cell bank

A cell bank is a collection of appropriate containers, whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of cells.

Cell line

Type of cell population which originates by serial subculture of a primary cell population, which can be banked.

Continuous cell line

A cell line having an infinite capacity for growth. Often referred to as "immortal" and previously referred to as "established".

Diploid cell line

A cell line having a finite *in vitro* lifespan in which the chromosomes are paired (euploid) and are structurally identical to those of the species from which they were derived.

Host cells

See Parental cells.

***In vitro* cell age**

Measure of time between thaw of the MCB vial(s) to harvest of the production vessel measured by elapsed chronological time, by population doubling level of the cells, or by passage level of the cells when subcultivated by a defined procedure for dilution of the culture.

Metazoan

Organism of multicellular animal nature.

MCB (Master Cell Bank)

An aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a new MCB (from a previous initial cell clone, MCB or WCB) should be the same as for the MCB unless justified.

Parental cells

Cell to be manipulated to give rise to a cell substrate or an intermediate cell line. For microbial expression systems, it is typical to also describe the parental cells as the host cell. For hybridomas, it is typical to also describe the parental cells as the cells to be fused.

WCB (Working Cell Bank)

The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.

APPENDIX 1: PRIMARY CELL SUBSTRATES

I. Introduction

The principles contained in this document apply in general to biotechnological/biological products prepared from characterized banked cells. However, a number of biological products, in particular certain viral vaccines, are prepared using primary cells.

Because primary cell cultures are used within the first passage after establishment from the tissue of origin, it is not possible to carry out extensive characterization of the cells prior to their use as is done for banked cell substrates. In addition, biological products produced using primary cell substrates often do not undergo extensive processing (e.g., purification). Despite these differences, the approach taken to assure the suitability and safety of primary cell substrates for production of biologicals is analogous, in many respects, to that outlined in this document and in other guidelines.

This Annex outlines cell substrate-related information that should be included in marketing applications for biological products prepared using primary cells. This information falls into three general categories: (1) Information concerning the source tissue (or organ) and other animal-derived raw materials used for the establishment of primary cell substrates, (2) information concerning the preparation of primary cell substrates, and (3) testing performed on primary cell substrates to ensure the safety of the product.

II. Source Tissue and Other Raw Materials

Information should be provided about the animals used as a source of tissue for the preparation of primary cell substrates. Tissue should be derived from healthy animals subjected to veterinary and laboratory monitoring to certify the absence of pathogenic agents. Whenever possible, donor animals should be obtained from closed, specific pathogen-free (when available) colonies or flocks. Animals used as tissue donors should not have been used previously for experimental studies. Animals should be adequately quarantined for an appropriate period of time prior to use for the preparation of cells. In some countries, animals may need to be quarantined in the country where the primary cells are prepared. Manufacturers should consult with national/regional authorities for specific requirements.

Information on materials and components used for the preparation of primary cell substrates should be provided, including the identity and source of all reagents of human or animal origin. A description of testing performed on components of animal origin to certify the absence of detectable contaminants and adventitious agents should be included.

III. Preparation of Primary Cell Substrates

Methods used for isolation of cells from tissue, establishment of primary cell cultures and maintenance of cultures should be described.

IV. Testing of Primary Cell Substrates

Tests performed on primary cell substrates to qualify them for use in production should be described. As noted, the nature of primary cell substrates precludes extensive testing and characterization prior to use. Testing to demonstrate the absence of adventitious agents in these substrates is therefore conducted concurrently and may include: Observation of production or uninfected control

cultures before, during, and beyond the period of production; inoculation of culture fluids from production and uninfected control cultures into various susceptible indicator cell cultures capable of detecting a wide range of relevant viruses, followed by examination for cytopathic changes and testing for the presence of hemadsorbing viruses; and other tests for specific agents (such as relevant retroviruses) as necessary. Additional information concerning specific viral tests may be found in the relevant national/regional/international guidelines.

Appropriate testing regimens and test methods for cells used in the production of specific products will vary depending on the donor species used as a source of tissue, adventitious agents potentially present, the nature of the product, its intended clinical use, aspects of the manufacturing process, and the extent of testing performed on the final product. Applicants should explain and justify the approach taken with respect to their specific product.